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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of:
Joanne Y. H. Kwak-Kim et al.

For: DIAGNOSIS AND TREATMENT OF INFERTILITY

Serial No. 10/651,690

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CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the United States Postal Office with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 23, 2006.

Tin-Chuen Yeung

COMBINED DECLARATION OF JOINT INVENTORS UNDER 37 C.F.R. §131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Joanne Young Hee Kwak-Kim, M.D. and Alice Gilman-Sachs, Ph.D. aver as follows:

1. We are over the age of twenty-one years and make these statements from our own personal knowledge.

2. I, Dr. Kwak-Kim currently hold the position of the Assistant Chair, Department of Obstetrics and Gynecology; and the Medical Director, the Clinics at Rosalind Franklin University of Medicine and Science; and the Director, Women's Health Division, University Clinics; and Associate Professor, Department of Obstetrics and Gynecology and the Department of Microbiology and Immunology of the Rosalind Franklin University of Medicine and Science (formerly known as Finch University of Health Sciences)/The Chicago Medical School.

3. I, Dr. Gilman-Sachs, currently hold the position of Associate Professor of the Rosalind Franklin University of Medicine and Science (RFUMS) and also hold the position of Associate Director Clinical Immunology Laboratory for RFUMS.

4. We are both joint inventor of the above-captioned patent application.

5. Joint inventor Alan E. Beer is deceased.

6. Prior to April 19, 1999 we planned to study the affect on reproductive outcomes, in subjects with a history of recurrent spontaneous abortions or implantation failures, by adjusting the balance of T helper 1 (Th1) and T helper 2 (Th2) immune responses in the subject. A letter signed by Dr. Kwak-Kim with the date expurgated is attached as Exhibit 1 and was mailed prior to the Critical Date. In particular, we determined to decrease the ratio of Th1 immune response to Th2 immune response by either (a) down regulating the Th1 immune response, (b) by up regulating the Th2 immune response or (c) by both down regulating the Th1 immune response while up regulating the Th2 immune response.

7. Further to this planned study, prior to the Critical Date we began development of an assay to measure the ratio of Th1 to Th2 immune responses in a subject. We have attached as Exhibit 2 a set of laboratory notebook pages with dates removed evidencing the development of the assay. The ratio of the Th1 to Th2 immune responses can be measured by absolute cell counts or percentage of Th1 cells to Th2 cells. Th1 cells are the activated T-cells expressing Th1 cytokines such as IL-1, IL-2, IFN-γ and TNF-α. Th2 cells are the activated T-cells expressing Th2 cytokines such as IL-4, IL-5, IL-6 and IL-10. The ratio of the Th1 to Th2 immune responses can also be determined by calculating a ratio of any one of the Th1 cytokines to any one of the Th2 cytokines.

8. One method we contemplated to reduce the Th1 count was to administer to a subject, prior to conception by the subject, a TNF-α antagonist. TNF-α antagonist may be of several types including antibodies, soluble receptors, and chemical compounds. We contemplated using several commercially available TNF-α antagonists and TNF-α antagonists that were undergoing an FDA approval process in the hope of becoming commercially saleable. Examples of antibody type and soluble receptor-type TNF-α antagonists included, but were not limited to: (1) infliximab (antibody-type) (2) entanercept (soluble receptor-type) (See Exhibit 1), (3) D2E7 (antibody-type) (4) CDP571 (antibody-type) and (5) CDP870 (antibody-type).

9. We contemplated administering the TNF-α antagonist by any medically suitable route of administration.

10. After conceiving of these concepts we worked on them diligently from prior to the Critical Date up to the time of filing the above-captioned patent application.

11. All of the work we have referred to herein was done in the United States of America.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, I acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and may jeopardize the validity of the application or any patent issuing thereon.

Date: 5/17/2006 BY Joanne Kwak-Kim

Dr. Joanne Kwak-Kim

Date: 5/17/2006 Alice Gilman-Sachs

Dr. Alice Gilman-Sachs

EXHIBIT 1

Mr. Richard McKenna
Medial Scientist Liaison
Wyeth-Ayerst Laboratories
15060 Hale Drive
Orland Park, IL 60462

Dear Mr. McKenna:

Thank you for your prompt response. I was glad to hear that your company had an interest in possible anti-TNF application for women with recurrent spontaneous abortions and infertility of immune etiology. I am sending some of our research articles and patient education materials for your perusal. You may find other information in our web site, repro-med.net.

I am preparing my idea for a possible clinical study using etanercept. Hopefully we can conduct a nice clinical trial in future.

If you have any questions, please feel free to contact me at any time.

Sincerely,

Joanne Kwak-Kim, M.D.

Joanne Y. H. Kwak-Kim, M.D.
Associate Director, Reproductive Medicine
Assistant Professor, Department of Obstetrics and Gynecology
Assistant Professor, Department of Microbiology and Immunology

EXHIBIT 2

Signal plot

~~* select the column~~

① Statistics

② Regression wizard

③ Sigmoidal ~~3 parameter~~

④ Logistic 4 parameter

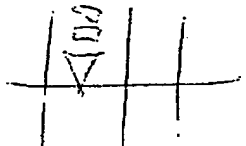
(if higher parameter does not work, use lower one (3))

⑤ parameters: First empty
need in report ☑
create new graph ☑

↓

Finish.

* File or column



type

* folder list or name change

Rt click : Edit click

→ type new name

1) IgG 1mg/ml 50ul 1hr
2) Block 200ul 1% BSA
3) Enz conj anti-IgG 50ul
Substrate 50ul

50ul

IgG (4g dilute)

1:10 1:10 1:100 1:100 1:500 1:500 1:1000 1:1000

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	IgG	1:10	1:100	1:100							
B		1:10	1:10									
C		1:10	1:10									
D		1:10	1:10									
E		1:10										
F		1:10										
G												
H												

(5) (ml)
1 ~ 1000 ml
0.1 ~ 100
0.01 ~ 10
0.005 ~ 5ml

1:1000
"
1:2000
"
1:4000
"
2ml + 2ml PBS
0.1% BSA-PBS

50ul

12x 0.05ml (1ml) PBS

1:10 1ml 0.1ml + 0.9ml PBS
1:100 1ml 50ul + 4.95 ml
1:500 1ml 0.2ml + 0.8ml
1:1000 1ml 0.1ml (100) + 0.9ml

0.2ml = 200ul.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B												
C												
D	•											
E												
F												
G												
H												

end + substrate = yellow

1) Antigen — 1 hr to overnight in bicarbonate buffer of pH 9.0 (sticky)

2) Wash x 4 add blocking reagent 1% BSA and PBS (phosphate buffered saline)
↳ 1 hr wait

3) Add Antibody (human serum)
↳ 1 hr wait

4) Wash.

5) Add indirect enzyme conjugate Ab
↳ 1 hr wait

6) Wash → Add substrate

Ag { 1:100 (bicarbonate) ↓ 20 µl + 2 ml
1:500 (") ↓ 200 µl + 0.8 ml
1:1000 (") 200 µl of 1:100 + 1.8 ml bicarbonate
1:2000 (") 0.05 ml (50 µl of 1:100) + 1.0 ml bicarbonate

human FSC in bicarb

1:100 1:100 1:500 1:500 1:1000 1:1000 1:2000 1:2000

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank											
B	BSS											
C												
D												
E												
F												
G						NOTH						
H												

A 1:2000
1:4000
1:2000
NOTH
1:4000

Ag * 1:100 → .1 v 0.0
.01 → 1 ml (20 µl + 2 ml) 12 x 0.05 ml = .60 ml

* 1:500 1:5 dil = 1:500 0.1 ml v 0.4
0.2 ml v 0.8

1:1000 dil 0.1 ml + 0.9 ml (0.2 ml + 1.8)
1:2000 dil ⇒ 1:20 dil 1 → 20 or .1 → 2.0

0.1 → 2.0 ml
0.05 → 1 ml
0.05 → 1.0 ml

① Eliza : basic concept.

Ag (thyroglobin) Anti Ab

Block + Pt's Serum (1:50 dilution
(1% Bovine serum albumin) (Anti Ab to thyroglobin)
{ 1ml → 50ml
0.1ml → 5ml
0.01ml → 0.5ml
(10ul → 500ul)

Ig (thyroglobin)
(Ag)

(goat anti human IgG
AP: Alkaline phosphatase
(human)

(2)

IgG
Alk phosph anti human
IgG
substr

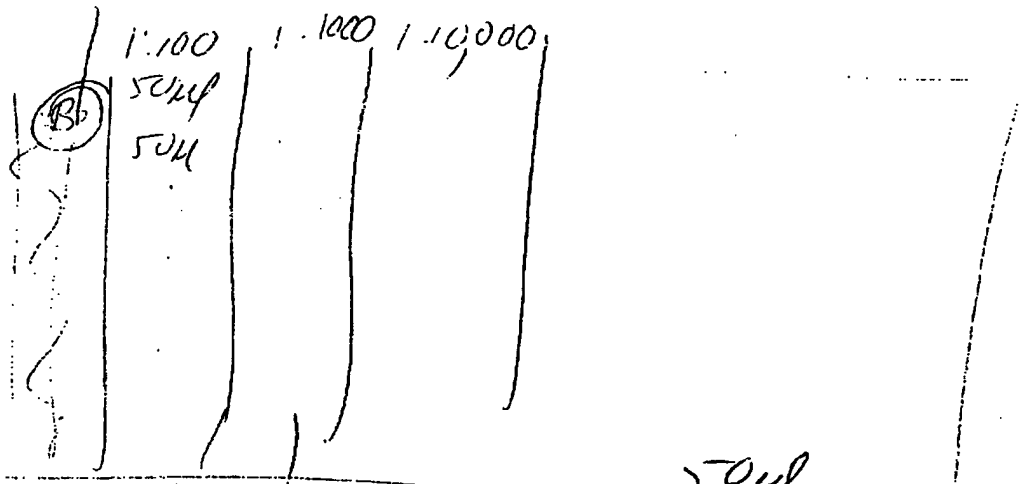
IgG
1000 µg/ml in PBS

1: 100 = Bicarbonate buffer.

1: 1000

1: 10,000

Bicarb 50µl



50µl

Leave on 1 hr RT

Wash 4x = PBS-tween 20 0.2% 200µl
Block with 1% BSA Hr / hr (200µl)
Dump
Add ~~conte~~ AP anti-human IgG (50µl) 1hr
in 0.1% BSA
Wash 4x = PBS-Tween 20 (200µl)
Add substrate (50µl) 30min 37°C
Add stopping reagent (optional)
Read OD

1 ml : 199 ml PBS

① anti-CD Ab ( ) mg/ml
→ dilute 1:200 in PBS [No BSA, No Serum],
place 30 ul in each well for stimulation.

② Incubate 1 hr at 37°C or
Overnight at 4°C (refrigerator)

③ Wash cells 2 x with 200 ul PBS - tap out on paper towel (should be sterile)

④ * No stim cells at least 1" away from stim

stim	N	
●	○	○
●	○	○
●	○	○
●	○	○

⑤ 200,000 cells/well in 200 ul
(= 1×10^6/ml)

⑥ for flow, Centrifuge in regular tubes, put supernatant into eppendorf.

⑦ collect S/N at 24 hrs, centrifuge in microfuge and place in a fresh tube

⑧ Assay by ELISA immediately or
freeze S/N ≤ -20°C

if multiple ELISA; Aliquot S/N.

— Read ELISA protocol ahead of time
How much sample do you need?

IL-10 1:10 → 100 ul

PHA (24 hr) + αCD3] compare
− αCD3

⊛ R&D systems
DO NOT USE BIOSOURCE

PBMC ← Anti CD3 stim (pre?)

IL-2 ↓ (IFNγ) TH1
IL-10 ↑ TH2

10 ml women
10 preg women

anti CD3 10 ug/ml

αCD3 clone 2 mg/ml in PBS [NO BSA NO SERUM]

① dilute (1:200), place 30 µl in each well for stimulation

② incubate (1 hr at 37°C or overnight at 4°C (refrig.)

③ wash wells 2x with 200µl PBS (sterile) - tap out on paper towel.

④ 200,000 cells/well in 200µl = 1×10⁶/ml → 24 hr incubation (37°C CO₂)

* no stim wells at least 1 away from stim

5a for flow, c.fuge in regular tubes, put S/N into eppendorf

⑤ collect (S/N) at 24 hrs centrifuge in microfuge and place in a fresh tube.
→ collect the supernatant ↓ Eppendorf pipette

⑥ assay by ELISA immediately or

freeze S/N ≤ -20°C
if multiple ELISA's, aliquot S/N

- read ELISA protocol ahead of time
HOW MUCH SAMPLE DO YOU NEED?

Coulter Epics (Turn On)

① Computer Power On → (wait 20 min)

② 맨 윗칸 뚜껑 (가운데 검은 box) – orange line 중심
~~En~~ · waste box check – ½ 이상이면 dump
(2 white bottle – dry 해야
(2 Transparent bottle – ⅓ 이하 해야
· Orange 읽어서 → (기록)

③ Panel → Select → start up click & okay click

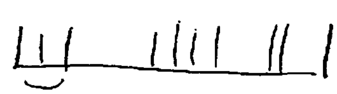
④ 뚜껑 맨 밑칸 Run 눌러 green blink 되면
open the door (옆의 긴 drawer)
→ Isoflud 채우고
button 2-3번 눌러 bubble 없는것 check

⑤ 화면에 Error message – click
Cleanse Error – click

⑥ Carrossal 에 3개 tube
① Water 1 ml 정도
② F-check : 10 drop
③ F-Set : 10 drop

⑦ 뚜껑 Run 눌러 initialization orange 되게 만듦

⑧ Insert tube 화면 뜨면
okay click → 5-7분 wait

⑨ Flow-check 정밀 : 
same
Flow-Set "

HPCV = CV
MnIX = Mean CH
MnX = Peak CH Copy 함

⑩ Protocol → Select.

< PCR 에 입력 >

Arg or Listmode
region - create
color click (colorful 사이 [illegible])
⇓
File
↓
PCX File

box 크기 조절해서 PCR에 연결되는 숫자를 사진을
저장시키도록.
(왼 mouse button 누른 채로 drag → rt click 하면)
⇓
PCX File
D:\ (하드 drive에 [illegible])
C:\XL\DATA\CD56.PCX

(다른 protocol로 Data를 불러 [illegible])

Listmode [Runtime protocol]

→ New protocol / panel

Shutdown } about 1ml
① water
② ~~two~~ bleach
③ water
④ water

panel
→ select
→ shut down
tube
→ Run (~~tube~~ 8-10 min) 끝나면 Run botton → green → push botton
(Manual dump) → it will be blinking
put the water 2X in manual tube
black tube 에 (양쪽 다 채움)
→ I can open the door
green + blinking (앞으로 약간 기울이면서 뺌)
→ take out ~~test~~ tube
→ black tube 넣음
→ black tube 넣음
→ 다른 black tube
→ test tube 다시 넣음
Auto mode procedure water ①, ②
put 2 tube
carousel 에 넣음
→ Auto → 동시에 누름
Cleanse

CD45 FITC / CD14 PE

CD3/CD4

CD3/CD8

CD5/CD16

CD3/IL2R

CD56/CD16

Cytokines

IL-1

IL-2

IL-3

IL-20

Target

NK : Effector

50:1

50K cells a sample

target

100,000 Target : 50:1

50K 100,000 Lymphocytes

1×10^6

$0.5 \times 100{,}000$

2.5×10^6

E:T

50:1

10%

2 hrs

effector

mediated

killing

E:T

25:1

5%

E:T

12.5:1

5%

Propidium iodide binds DNA

Sample #1

1500

100

10%

Sample #2

1000

900